

## REMARKS

In the Office Action dated May 26, 2010, claims 12-20 are rejected under 35 U.S.C. §103(a) as being allegedly unpatentable primarily over Bao et al (20030129611, July 2003) in view of Yoshimoto et al. (Chemical Communication, Issue 24, pages 2960-2961, October 2003). A Request for Continued Examination is submitted herewith. A Petition for a Two Month Extension of Time is also submitted herewith. The Commissioner is hereby authorized to charge deposit account 02-1818 for the RCE fee, the Extension of Time fee, and for any other fees which are due and owing. Applicants respectfully maintain disagreement with the rejections, and provide additional support for this position below.

### Bao

Bao relates to nucleic acid probes using the resonance energy transfer moieties. Bao shows that detecting the interaction between two molecular beacons hybridized on a subject nucleic acid using fluorescent detection by FRET or LRET.

In Bao, it is mentioned that detecting the target nucleic acid using the fluorescence resonance energy transfer by hybridization of the two kinds of the nucleic acids (molecular beacons) which are complementary to each of the target nucleic acid and the partial sequence of the target nucleic acid.

However, Bao requires performing the chemical modification of the attachment of the fluorescent material. That is, Bao discloses the following in paragraph [0045]:

“Probe and Target Design. All oligonucleotide probes were designed to be complementary in antisense orientation to the human GAPDH gene, as illustrated in FIG. 2. Specifically, a dabcy1 quencher was attached to the 5'-end and a 6-Fam fluorophore was attached to the 3'-end of donor molecular beacons; a dabcy1 quencher was attached to the 3'-end and either a Cyanine 3 (Cy3), 6-carboxyrhodamine (ROX), or Texas Red fluorophore was attached to the 5'-end of acceptor molecular beacons. The stem sequence was designed to participate in both hairpin formation and target hybridization (Tsourkas et al., 2002b).”

As mentioned above, the technology in Bao requires the attachment of the

fluorescent material to the two kinds of molecular beacons of the donor beacon and the acceptor beacon. Accordingly, in such technology, it is necessary that complicated operations for detecting the target base and cost becomes higher by attachment of the fluorescent material. Therefore, detecting the gene mutation with ease and low cost cannot be realized.

### Yoshimoto

Further, the technology described in Yoshimoto relates to a nucleic acid base using DNA chain containing AP-site. This provides for a method for fluorescence detecting of mutation in a target nucleic acid by hydrogen bond forming small compounds.

However, the technology in Yoshimoto requires performing the chemical modification substantially:

“Figure 1, an AP site containing DNA strand is hybridized with a normal DNA strand so as to place the AP site opposite from, but facing toward, a target nucleobase, by which hydrophobic microenvironments are provided for ligands to recognize nucleobase through hydrogen bonding,” (see, Yoshimoto, lines 4-8, second paragraph); and

“In this study, a tetrahydrofuranyl residue (dSpacer) which lacks a nucleobase moiety is utilize for design of an AP site and is incorporated with 11-mer oligodeoxynucleotides (5'-TCCAGXGCAAC-3', X=sSpacer, A=adenine, C=cytosine, G=Guanine, T=thymine),” (see, Yoshimoto, lines 11-15, second paragraph).

As mentioned above, it is evident to use an AP site which is a single stranded DNA containing special part, from the description of Yoshimoto. Accordingly, in the recognition of the nucleic acid base which Yoshimoto discloses, a special part such as a basic site(AP-site) needs to be introduced in the double stranded DNA in a complete aqueous solution, which corresponds to the chemical modification in a strict sense.

In this manner, the technology described in Yoshimoto also requires

construction of AP-site which corresponds to the chemical modification. In Yoshimoto, although marking the detecting DNA by a fluorescent material is not required, the complicated operation such as introducing the AP-site in the detecting nucleic acid is required. Thereby, a cost necessary when the detecting DNA is synthesized is high. Accordingly, it is not possible to realize detection of gene mutation in a simple and cost effective manner.

#### **Summary of the Presently Claimed Invention**

On the other hand, according to the presently claimed invention, forming a gap part at a position opposed to a target base by forming a double-stranded nucleic acid from: (i) a single-stranded target nucleic acid comprising a sequence of bases that includes the target base composed of one or more continuous bases and two partial sequences of bases with the target base there between; and (ii) two single-stranded detecting nucleic acids complementary to the two partial sequences of bases, wherein the single-stranded detecting nucleic acid form the gap part opposed to a target base on the single-stranded target nucleic acid; forming a hydrogen bond from the target base and a receptor by inserting the receptor having hydrogen bonding characteristics into the gap part; and identifying the gene mutation where the receptor bonds to the target base. That is, the gap part is formed intentionally in a part facing to the target base, and a receptor having hydrogen bonding characteristics is inserted into the gap part, a hydrogen bond is formed by the target base and the receptor, to identify the target base.

According to the presently claimed invention, neither the chemical modification such as attachment of the fluorescent material nor the chemical modification such as introducing previously a special part such as AP site in the single stranded target nucleic acid is necessary. Therefore, it is possible to form the gap part intentionally in a part facing to the target base and detect the target base facing the gap part without the chemical modification, so that it is possible to suppress a rise of cost when detecting DNA is synthesized, and it is possible to realize detection of a gene mutation simply and cost effective since the complicated operation that the AP-site is introduced is not required.

**Differences Between the Presently Claimed Invention and the Cited Art**

As mentioned above, it is impossible to arrive at the present application without requiring the chemical modification of the technology disclosed in Bao and Yoshimoto. Further, the technological fields of Bao and Yoshimoto are distinct, and it would not be obvious to one of skill in the art to combine the technologies of same. In this regard, it is not possible to arrive at the presently claimed invention without chemical modification.

The difference of the presently claimed invention and Bao is not only in the method for detecting the target base, but also in the technical concepts. As mentioned above, the technology in Bao relates to the nucleic acid probes using the resonance energy transfer moieties, and it is shown to detect the target base by the effect of the resonance energy transfer which is improved by fixing "a relative distance" between the donor and the acceptor beacons. This is explained in further detail below.

According to paragraph [0145] of Bao:

"This beacon design was chosen to help fix the relative distance between the donor and acceptor fluorophores and improve energy transfer efficiency.

Both the donor and acceptor beacons were designed with a probe length of 18 bases and a stem length of 5 bases. The probe length is defined as the portion of the molecular beacon that is complementary to the target. The synthetic wild-type GAPDH target has 4-base gap between the donor dye and the acceptor dye. Gap spacing was adjusted to 3, 5 and 6 bases by either removing a guanine residue or adding 1 or 2 thymine residues, as shown in Table 1."

It shows that the technology in Bao detects the target nucleic acid using the fluorescence resonance energy transfer which is improved by fixing "a relative distance" between the donor and the acceptor beacons. In other words, the detection strength depends on the effect of resonance energy transfer based on the relative distance between the two molecular beacons. The relative distance is adjusted by either removing a guanine residue or adding 1 or 2 thymine residues, and the detection strength is improved.

Accordingly, the gap spacing between the two molecular beacons affects to the effect of the resonance energy transfer. Therefore, adjusting the effect gives big technical definition to forming the gap.

Therefore, as shown in Fig. 2 of Bao, it is required to include not only the part facing the target base (G/A) but also the part facing the bases (A and T of 5' side of G/A) which are adjacent to the target base as a gap. In this way, the relative distance between the donor beacon and the acceptor beacon is adjusted by including to the gap the part facing the base which is not the target adjacent to the target base.

Here, the Examiner's is believed to be as follows. If the method for detecting in accordance with the receptor described in Yoshimoto is combined simply to Bao which the part facing the base which is not target adjacent to the target base includes in gap, the part facing the base which is not the target base become the gap. Thereby, the possibility occurs that the inserted receptor is inserted and to be combined in the part facing the base which is not the target base. In this case, it is not possible to perform highly precise detecting the target base which is a detection object.

To begin with, the above mentioned case occurs because Bao is the following technology: detect the target nucleic acid using the fluorescence resonance energy transfer which is improved by fixing "a relative distance" between the donor and the acceptor beacons. Therefore, Yoshimoto is not properly combinable with Bao. Moreover, even assuming that Bao was properly combinable with Yoshimoto, the references still fail to disclose or suggest each of the elements of Claim 12 and dependents thereof.

### **The Present Application**

On the other hand, according to the presently claimed invention, forming a gap part at a position opposed to a target base by forming a double-stranded nucleic acid from: (i) a single-stranded target nucleic acid comprising a sequence of bases that includes the target base composed of one or more continuous bases and two partial sequences of bases with the target base there between; and (ii) two single-stranded detecting nucleic acids complementary to the two partial sequences of bases, wherein the

single-stranded detecting nucleic acid form the gap part opposed to a target base on the single-stranded target nucleic acid: forming a hydrogen bond from the target base and a receptor by inserting the receptor having hydrogen bonding characteristics into the gap part; and identifying the gene mutation where the receptor bonds to the target base.

That is, the presently claimed invention discloses that the gap part is formed intentionally in a part facing to the target base, and the gap is not formed at the part facing the base which is not the target.

In this way, the technology recited in the presently claimed invention is distinguishable from the technology of Bao. Therefore, it is not possible for Bao to detect the target base at a high accuracy as in the presently claimed invention, even if the technology disclosed in Yoshimoto is combined with the technology of Bao.

### **Conclusion**

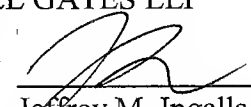
As mentioned above, there is significant difference concerning the existence or nonexistence of the chemical modification in the present application. The differences between Bao and Yoshimoto and the presently claimed invention results in a large difference in operability or cost for detecting the target base. Moreover, because the presently claimed invention is distinguishable from the technology of Bao in a technical concept, it is impossible for Bao to detect the target base in a high accuracy like the present application, even assuming that Yoshimoto is properly combinable with Bao.

Accordingly, Applicants believe the rejections should be withdrawn at least for these reasons and further submit that the present application is in condition for allowance.

Respectfully submitted,

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